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Genomes at the interface between bacteria and organelles

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The topic of the transition of the genome of a free-living bacterial organism to that of an organelle is addressed by considering three cases. Two of these are relatively clear-cut as involving respectively organisms (cyanobacteria) and organelles (plastids). Cyanobacteria are usually free-living but some are involved in symbioses with a range of eukaryotes in which the cyanobacterial partner contributes photosynthesis, nitrogen fixation, or both of these. In several of these symbioses the cyanobacterium is vertically transmitted, and in a few instances, sufficient unsuccessful attempts have been made to culture the cyanobiont independently for the association to be considered obligate for the cyanobacterium. Plastids clearly had a cyanobacterial ancestor but cannot grow independently of the host eukaryote. Plastid genomes have at most 15% of the number of genes encoded by the cyanobacterium with the smallest number of genes; more genes than are retained in the plastid genome have been transferred to the eukaryote nuclear genome, while the rest of the cyanobacterial genes have been lost. Even the most cyanobacteria-like plastids, for example the 'cyanelles' of glaucocystophyte algae, are functionally and genetically very similar to other plastids and give little help in indicating intermediates in the evolution of plastids. The third case considered is the vertically transmitted intracellular bacterial symbionts of insects where the symbiosis is usually obligate for both partners. The number of genes encoded by the genomes of these obligate symbionts is intermediate between that of organelles and that of free-living bacteria, and the genomes of the insect symbionts also show rapid rates of sequence evolution and AT (adenine, thymine) bias. Genetically and functionally, these insect symbionts show considerable similarity to organelles.

Keywords: *Buchnera*; cyanobacteria; gene transfer; plastids; *Wolbachia*

1. INTRODUCTION

This paper addresses some aspects of the transition from free-living bacteria to bacterial-derived organelles. We define a bacterial-derived organelle as an intracellular derivative of a symbiotic bacterium with transfer from the symbiont to the nucleus of one or more genes whose product(s) is (are) targeted back to the organelle. We consider free-living and symbiotic cyanobacteria, which are clearly organisms, even if in some cases of symbiotic cyanobacteria the symbiosis is obligate as far as the cyanobacterium is concerned in that attempts to culture the cyanobiont independently of the hosts have failed. At the other extreme we consider plastids, which are clearly bacterial-derived organelles with much less than half of the genes needed for functioning of the organelle retained in the plastid genome, the remainder having been transferred to the host nucleus. There are no known cases of intermediate stages in the evolution of plastids from a cyanobacterium, and the case of the cyanelles of glaucocystophyte algae, which are frequently mentioned in the context of early stages in the evolution of plastids, is critically discussed.

We also consider the case of intracellular bacteria in insects. These obligate symbionts (e.g. *Buchnera*) have characteristics that appear to be intermediate between those of bacteria and of bacteria-derived organelles. However, we shall see that the evidence on the lack of transfer of gene(s) from the symbiont to the host nucleus, with targeting of the gene product back to the symbionts, is incomplete.

2. FROM CYANOBACTERIA TO PLASTIDS: CROSSING THE INTERFACE BETWEEN BACTERIA AND ORGANELLES

(a) *Free-living and symbiotic cyanobacteria*

Free-living cyanobacteria are involved in photosynthesis and (for some taxa) nitrogen fixation on land and, more particularly, in inland waters and oceans. Cyanobacterial symbioses also involve photosynthesis and/or nitrogen fixation. All five of the morphological groups of cyanobacteria (Rippka *et al.* 1979) are involved in symbioses with eukaryotes (Liaimier 2002), and a very wide range of eukaryotes are the other partner in the symbioses, including diatoms, zygomycetous, ascomycetous and basidiomycetous fungi, sponges, ascidian hemichordates and embryophytic (higher) plants (Smith & Douglas 1987;

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Table 1. Metabolic inputs by cyanobionts to symbioses with eukaryotes (from Smith & Douglas 1987; Douglas 1994; Rai *et al.* 2000; Raven 2002*a*,*b*).
(Abbreviations: e, extracellular; i, intracellular.)

host, and location of cyanobionts	cyanobiont	photosynthesis by cyanobiont *in hospice*	diazotrophic N supply by cyanobiont *in hospice*
non-photosynthetic marine Porifera (e, i), echiuroid worms (e) and didemnid acidians (e)	unicellular or non-heterocystous filaments	yes, whenever tested	yes, in some Porifera and (possibly) ascidians
non-photosynthetic member of Zygomycotina (i; *Geosiphon*)	heterocystous filaments	yes	yes
non-photosynthetic Ascomycotina without symbiotic green algae (e); non-photosynthetic Basidiomycotina (e)	unicells or heterocystous filaments	yes	yes
photosynthetic marine and freshwater diatoms (e; within cell wall, apparently outside plasmalemma).	heterocystous filaments or unicells	minor contribution?	yes, in marine; possible in freshwater
Ascomycotina with green algal photobionts (e), liverworts (*Blasia*, *Cavicularia*; (e), many hornworts (e), few mosses (e), pteridophyte *Azolla* (e), cycads (e, i), angiosperm *Gunnera* (i)	heterocystous filaments	minor contribution in all but cycads and *Gunnera* where the cyanobionts are in the dark	yes

Table 2. Cases of vertical transmission of cyanobionts (from Smith & Douglas 1987; Douglas 1994; Rai *et al.* 2000; Raven 2002*a*,*b*).

host	cyanobiont location	mechanism of vertical transmission of cyanobiont
diatoms	enclosed by frustules but not truly intracellular	with vegetative cell division and (presumably) sexual reproduction
Porifera (some)	intracellular	in oocytes and nurse cells
didemnid acidians	extracellular	via a 'plant rake' and 'algal pouch'
lichens	extracellular	in soredia (assexual propagules)
Azolla	extracellular	via megasporocarps and megaspores

Douglas 1994; Rai *et al.* 2000; Liaimier 2002; Raven 2002*a*,*b*). Most of the symbiotic cyanobacteria that have been examined in detail are capable of free-living existence. However, this is not essential even if the cyanobionts are not vertically transmitted as they could be transmitted contagiously (see below). The much-investigated *Nostoc* symbiont of the heterosporous water fern *Azolla* has not yet been cultured, but is vertically transmitted.

Three important aspects of cyanobacterial symbioses with eukaryotes are the metabolic contribution made by the cyanobiont (table 1), the intracellular or extracellular location of the cyanobiont (table 1) and the means by which each new generation of host acquires symbionts (table 2).

Table 1 shows the metabolic contribution of the cyanobionts to the symbioses. All symbioses which lack other photosynthetic components involve photosynthesis by the cyanobiont; the cyanobionts generally convert chemoorganic hosts into photolithotrophic symbioses. When the symbiosis involves hosts that are already photosynthetically competent by means of plastids or, in some ascomycetous lichens, green algae, the cyanobionts contribute N_2 fixation (table 1).

There is sometimes N_2 fixation associated with cyanobacteria involved with non-photosynthetic hosts (table 1). Similarly, N_2-fixing cyanobacteria associated with photosynthetic hosts can make a (relatively minor) contribution to photosynthesis by the symbioses whenever the cyanobacteria are exposed to light.

If an extant cyanobacterial symbiosis is to be in any way analogous to the origin of plastids it must be photosynthetic and intracellular. The data in table 1 show that only sponges (Porifera) and *Geosiphon* are possibilities. The other criterion is vertical transmission which only coincides with intracellular cyanobionts in the case of sexual reproduction in some marine sponges (table 2). The relative rarity of vertical transmission among intracellular cyanobacterial symbioses is presumably explicable, granted more information, in terms of the evolutionary considerations described by Douglas (1998*a*) and Ferriere *et al.* (2002).

The data in tables 1 and 2 show that there are examples of vertical transmission of truly intracellular diazotrophic cyanobionts in some marine sponges, and of not truly intracellular cyanobionts in diatoms. There are no known cases of diazotrophic organelles, although the vertical transmission of intracellular diazotrophic cyanobacteria shows that the spatial barrier to vertical transmission of

Table 3. Genome size and number of genes in cyanobacteria, in the cyanelle of the glaucocystophyte *Cyanophora paradoxa*, and in other plastids ('euplastids').

genome	genome size (in kilobase pairs)	number of genes	reference
Synechocystis sp.PCC 6803 genome	3573	4003 ORFs	Kaneko *et al.* (1996)
Prochlorococcus MED 4 genome	1800	1686 ORFs	Hess *et al.* (2001)
Prochlorococcus MIT 9313 genome	2400	2200 ORFs	Hess *et al.* (2001)
Prohlorothrix hollandica PCC 9006 genome	5500	?	Schyns *et al.* (1997)
Nostoc punctiforme PCC 73102	> 9000	?	Meeks *et al.* (2001)
Cyanophora paradoxa UTEX 555 cyanelle	135.6	150 total	Löffelhardt *et al.* (1997*a*)
Cyanidium caldarium RK1 plastid	164.9	199 ORFs (33 RNA)	Glockner *et al.* (2000)
Porphyra purpurea plastid	191	154 ORFs; 255 total	Reith & Mulholland (1995)
Guillardia theta plastid	121.5	187 total	Douglas & Penny (1999)
Odontella sinensis plastid	119.7	174	Kowallik *et al.* (1995)
Mesostigma viride plastid	118.4	135 ORFs	Lemieux *et al.* (2000)
Nephroselmis olivacia plastid	200.8	127 ORFs	Turmel *et al.* (1999)
Chlamydomonas mouewsii plastid	292	?	Reith (1995)

rhizobia and *Frankia* diazotrophic symbionts of higher plants (Douglas 1994) is not a universal feature of diazotrophic symbioses. It is still not certain why there are no diazotrophic organelles (see Raven 2002*a*,*b*), especially as it is very possible that the cyanobacterial ancestor of plastids was a diazotroph (Martin *et al.* 2002).

Two further aspects of free-living and symbiotic cyanobacteria that need consideration before dealing with plastids are the nature of the genome of cyanobacteria, and the nearest extant cyanobacterial relative of the plastid ancestor.

The size and number of genes encoded (where known) in cyanobacterial genomes are shown in table 3. The genome sizes range from only 1.8 megabase pairs (Mbp) to over 9.0 Mbp and the smaller genomes (up to 3.6 Mbp) have between 1686 and 4003 ORFs. There is a clear discontinuity in size, and number of ORFs, between cyanobacteria and plastids listed in table 3, as discussed below.

The nearest extant cyanobacterial relative to the plastid ancestor (assuming a unique primary endosymbiosis event giving rise to all plastids; see below) is still a matter of some controversy, but it appears that plastids arose from near the base of the cyanobacterial clade (Turner *et al.* 1999). It is not even certain if the plastid ancestor was a diazotroph (Raven 2002*a*,*b*), although there are good arguments that it contained both phycobilins and chlorophyll *b* (Tomitani *et al.* 1999; Ting *et al.* 2002).

(b) *The origin of plastids*

Most molecular genetic evidence is consistent with a monophyletic origin of plastids, so that a single symbiotic (Mereschowsky 1905; Martin & Kowallik 1999) event involving a cyanobacterium and a mitochondria-containing eukaryote gave rise to all plastids (Stoebe & Kowallik 1999; Tomitani *et al.* 1999; Turner *et al.* 1999; Moreira *et al.* 2000; McFadden 2001; Martin *et al.* 2002; cf. Lockhart *et al.* 1992). On this hypothesis the plastids of rhodophyte, chlorophyte and glaucocystophyte algae are primary plastids derived from this single endosymbiotic event by radiation of the first plastid-containing symbiont, whereas all other plastids are the result of secondary or even tertiary endosymbioses (McFadden 2001) of green or red algal unicells and a range of phagotrophs. However, some evidence, based on the nucleotide sequence of the largest subunit of RNA polymerase II, favours divergence of the host (eukaryotic) nucleus of red algae before the origin of green algae (and hence higher plants), fungi and metazoa, and this divergence requires more than one primary endosymbiotic event (Stiller & Hall 1997, 1998; Stiller *et al.* 2001). Plastids other than those of rhodophytes, glaucocystophytes and chlorophytes (and their progeny, the higher plants) arose by multiple secondary endosymbioses (McFadden 1999, 2001). Although not necessarily conforming to the strictest of definitions of symbiosis, kleptoplastids in sacoglossan molluscs may have lessons for how secondary (and tertiary) endosymbioses occurred. These kleptoplastids, obtained from macroalgal food plants, function for up to eight months *in hospice* despite their plastid genomes lacking many of the genes needed for the replacement of plastid components (Green *et al.* 2000; Rumpho *et al.* 2000; Harten & Pierce 2001; Raven *et al.* 2001). For further discussion we shall accept the monophyletic origin of plastids.

(c) *The genome size and gene complement of plastids*

Table 3 gives the genome size and (where available) number of ORFs for several algal plastids. This history is not exhaustive, excluding many well-characterized algal plastid genomes and all higher-plant plastid genomes, but it does contain the largest-known plastid genome (*Chlamydomonas moewusii*) and the plastid genomes with the largest number of ORFs. It is clear from table 3 that, on the basis of present knowledge, there is a clear distinction between free-living (and potentially symbiotic in the case of *Nostoc*) cyanobacteria and plastids in terms of both genome size and number of ORFs. Since the loss of only one gene essential for independent growth from a cyanobiont is sufficient to render it an obligate symbiont, it is clear that plastids have gone well beyond this condition, with not more than 255 ORFs in plastids and not less than 1686 ORFs in free-living cyanobacteria.

The genes retained by the plastids are mainly concerned with the replication, transcription and translation of the plastid genome, and with producing and maintaining the photosynthetic apparatus (Allen & Raven 1996). However, a significant number of genes relating to both the genetic apparatus of the plastids and to photosynthesis have been transferred to the nuclear genome (Allen & Raven 1996). Furthermore, a very significant number of genes not related to photosynthesis or to plastid genome functioning has been transferred from the plastid ancestor to the nucleus. Martin *et al.* (2002) (cf. Rujan & Martin 2001; Palenik 2002) compared 24 990 proteins encoded in the *Arabidopsis* genome to proteins from three completely sequenced cyanobacterial genomes (*Nostoc, Prochlorococcus* and *Synechocystis*). The analysis indicates that about 4500 protein-encoding genes in *Arabidopsis*, or *ca*. 18% of the total, were acquired from cyanobacterial ancestor of plastids.

(d) *Factors that may determine whether genes from the plastid ancestor shall be retained in the plastid genome, transferred to the host nucleus, or lost from the symbiosis*

Several authors have considered these questions (e.g. Allen 1993*a*,*b*,*c*, 1995, 1996; Allen & Raven 1996; Allen & Nilsson 1997; McFadden 1999; Race *et al*. 1999; Blanchard & Lynch 2000; Howe *et al*. 2000; Saccone *et al*. 2000; Henze & Martin 2001; Rujan & Martin 2001; Selosse *et al*. 2001; Zerges 2000, 2002; Martin *et al.* 2002) which are fundamental to considerations of why plastid genomes have the genes that they do. The transfer of genes from organelles to the nucleus (with a much smaller transfer of genes from nucleus to organelles) is a continuing process whenever coding changes (e.g. in mammalian mitochondria) have not precluded it (Blanchard & Lynch 2000; Miller *et al.* 2001).

(i) *Possible reasons for plastid ancestor gene loss from the symbioses*

Considering first the reasons for loss of plastid ancestor genes from the symbiosis, several cyanobacterial genes code for products that are not required in the symbiosis or, if required, duplicate genes, already in the host nucleus. Rujan & Martin (2001) and Martin *et al.* (2002) point out that a significant minority of non-photosynthetic genes in plants were derived from the plastid ancestor, in some cases presumably replacing pre-existing nuclear genes. Even the genes for the peptidoglycan cell wall of the cyanobacterial ancestor are retained in some algae, as is discussed later with respect to 'intermediates' in plastid evolution. As far as is known all of the genes retained from the plastid ancestor whose products function in cellular locations other than the plastid are now in the nucleus.

(ii) *Possible reasons for the transfer of plastid ancestor genes to the nucleus*

As well as the genes from the plastid ancestor which relate to function not performed in plastids, most of the genes relating to processes that occur in the plastid have been transferred to the nucleus. These include many of the genes for thylakoid and stroma processes of photosynthesis as well as such processes as nitrite reduction. Selection pressures that could have favoured such transfer include the economics of maintaining several genomes rather than consolidation of all genes into the nucleus, and the removal of genes from the mutagenic environment in the energy-transducing organelles where redox reactions generate free radicals (Allen 1996; Allen & Raven 1996; McFadden 1999, 2001; Selosse *et al.* 2001).

The economic argument is that maintaining separate genomes with their attendant replications, transcription and translation apparatus is costly in resources. This is especially the case where there are distinct versions of, for example, ribosomes in the plastids and mitochondria and in the cytosol. The extreme case is a single remaining gene encoding a photosynthetically essential protein in the plastid genome; this single gene requires the full molecular genetic apparatus to be functional, just as is the case for 100 photosynthetically essential genes. Because all plastids retain genomes, this argument clearly does not counteract reasons for gene retention in plastids. This is not the case for the mitochondria/hydrogenosomes class of organelles where almost all hydrogenosomes lack a genome (Akhmanova *et al.* 1998; Hackstein *et al.* 2001).

The 'mutagenic environment' argument is explained in detail in Allen & Raven (1996). Essentially the reasoning is that the active oxygen species generated by redox reactions in the presence of O_2 damage the gene products and the genes, leading to an error catastrophe unless resource-expensive scavenging and quenching components are produced in greater amounts than in other cell compartments. This argument applies to photosynthetically active plastids, and to mitochondria functional in aerobic environments. To the active oxygen species considered by Allen & Raven (1996) can be added NO, which is much less toxic than hydroxyl radical or singlet oxygen. NO can be produced in eukaryotic photosynthetic organisms when there is an excess of nitrate reduction in the cytosol over nitrate reduction in the plastid, e.g. upon darkening (Mallick *et al.* 1999, 2000*a*,*b*,*c*; Galvan *et al.* 2002). NO could also be produced in dissimilatory nitrate reduction in the mitochondria of *Loxodes* in anoxic habitats (Finlay *et al.* 1983). The mutagenic free radical hypothesis does not explain the absence of genomes in most hydrogenosomes where no O_2 free radicals are produced.

The final argument used by Allen & Raven (1996) for relocation of plastid (and all organelle) genes to the nucleus is that organelle genes are almost always uniparentally inherited, and, at least as important, have a small effective population size and thus are subject to the operation of Muller's ratchet (see Allen 1996; Allen & Raven 1996). Muller's ratchet is the term used to describe the repeated loss of the most-fit genotypes in asexual populations, and applies particularly to small asexual populations of intracellular micro-organisms. Deleterious mutations increase in such genomes which lack the possibility of recombination. Genomic decay in plastids can lead to AT bias (Howe *et al.* 2000), although there is also selection for the user of codons involving abundant tRNAs in the case of highly expressed plastid genes such as *psbA* (Morton 1998, 2001). AT bias can also distort phylogenetic reconstructions of plastid ancestry (Lockhart *et al.* 1992). The Muller's ratchet argument is a powerful one, and is not matched by contrary suggestions, e.g. that the absence of recombination prevents the spread of deleterious genes through the population such as could occur for

nuclear genes in a sexually producing species. Blanchard & Lynch (2000) critique other population genetic implications of gene transfer to the nucleus.

(iii) *Possible reasons for the retention of plastid ancestor genes in the plastids*

The arguments for retaining genes in plastids (and other organelles) are that some genes yield protein products that could not readily be imported into the organelle, and that control of transcription of chloroplast (and mitochondrial) genes by the redox state of the organelle is a key regulatory feature which would be much more complex to manage for nuclear genes (see Allen & Raven 1996; Pfannschmidt *et al.* 2001*a*,*b*).

While the suggestion that some proteins cannot readily be transported into organelles has not been tested for all of the plastid- (or mitochondria-) genome protein-coding genes, it is clear from experimental and natural situations that it does not apply to the hydrophilic 55 kDa large subunit of RUBISCO (see Kanerski & Maliga 1994; Morse *et al.* 1995; Whitney & Yellowlees 1995; Whitney *et al.* 1995; Allen & Raven 1996).

The argument about redox control of transcription of certain organelle-encoded genes has not been falsified since Allen proposed it (1993*a*,*b*,*c*, 1995; Pfannschmidt *et al.* 1999, 2001*a*,*b*; Surpin *et al.* 2002), and it seems to be a strong argument for retention of the core of genes found in all known plastid (Allen & Raven 1996; Martin *et al.* 1998; Barbrook *et al.* 2001; references in table 3) and mitochondrial genomes. The list of universally plastid-encoded genes in Allen & Raven (1996) has proved to be robust, although the large subunit of RUBISCO is now known to be nuclear-encoded in peridinin-containing dinoflagellates (Morse *et al.* 1995). However, it is not obviously applicable to plastid genomes in organisms that lack photosynthetic function, e.g. the achlorophyllous parasitic flowering plant *Epifagus*, and the apicomplexans such as *Toxoplasma* and *Plasmodium*.

There is also a problem with the retention of the nucleomorph genome in cryptophyte and chlororachniophyte algae (McFadden 2001). These eukaryotic chromosomal structures are the remains of the nucleus of the green (for chlororachniophyte) and red (for cryptophyte) algae which contributed the plastids in the secondary symbiotic event which converted the phagotrophic ancestors of these algae into phototrophs (McFadden 2001). The nucleomorph chromosomes encode some proteins needed by plastids, but their location makes them almost as remote from transcriptional control by plastid redox state as nuclear genes in cryptomonads and chlororachniophytes (McFadden 2001).

To end on a positive, albeit non-plastid note, the strongly reducing conditions of life of hydrogenosome-containing organisms could be construed as arguing for the absence of significant redox potential changes in hydrogenosomes, rationalizing the absence of a genome in all but one of the hydrogenosomes investigated (Akhamanova *et al.* 1998; Hackstein *et al.* 2001).

(e) *Intermediate stages in plastid evolution?*

Many evolutionary biologists seem to be drawn to the search for 'missing links', and the origin and evolution of plastids are no exception. Much attention has focused on glaucocystophyte algae and the thecate amoeba *Paulinella* which, by having plastids (cyanelles or muroplasts) that are not only of similar pigmentation to many cyanobacteria but also have a peptidoglycan cell wall, could be considered as having plastids that are most like the plastid ancestors (Kies 1992; Löffelhardt *et al.* 1997*a*,*b*). We now consider the plastids of glaucocystophytes (Kies 1992; Bhattacharya *et al.* 1995; Helmchen *et al.* 1995) and of *Paulinella* (Kies 1992) in this context.

The plesiomorphic characters of cyanelles are the peptidoglycan wall and the carboxysomes (Kies 1992; Van den Hoek *et al.* 1995; Pfanzagl *et al.* 1996). We outline here a possible functional correlation between these two characters which is developed at greater length elsewhere (Raven 2003). All plastids have the FtsZ-based division mechanism of cell division of cyanobacteria and cyanelles, but it is only the cyanelles that have a peptidoglycan wall like cyanobacteria and other eubacteria (Beech & Gilson 2000). However, some green algal plastid genomes have FtsI, a homologue of peptidoglycan synthase (Turmel *et al.* 1999; Lemieux *et al.* 2000), and the division of some bryophyte plastids is inhibited by β-lactam antibiotics (Kasten & Reski 1997; Clauss *et al.* 2000). The peptidoglycan wall of bacteria is related to the development of turgor as a result of the higher osmolarity of the cell contents than of the growth medium (Koch 2001). Turgor is required for growth of bacterial, including cyanobacterial, cells (Koch 2001). It is likely that cyanelles are also hyperosmotic to their medium, the cytosol, unlike plastids which have no walls (Nobel 1975; cf. Larkum 1968; Grant & Borowitzka 1984*a*,*b*). The cyanelle-containing glaucocystophytes and *Paulinella* are all freshwater organisms, and all rely on cytosol volume regulation by active water efflux during at least part of their life cycle (Kies (1992) and references therein (cf. Raven 1982, 1995)). The intracellular osmolarity of freshwater cells relying on contractile vacuoles, or analogues that lack the structural characteristics of contractile vacuoles but can also catalyse the active efflux of water, for cytosolic volume regulation is often low (20–40 osmol m^{-3}) possibly for the energetic reasons discussed by Raven (1982, 1984, 1995). The intracellular osmolarity of freshwater cyanobacteria is 80–200 osmol m^{-3} (Walsby 1980, 1986).

The significance of a higher osmolarity in cyanelles than in the cytosol may relate to the functioning of carboxysomes. Carboxysomes in bacteria function in the inorganic carbon concentrating mechanism which increases the carbon dioxide concentration around RUBISCO (Badger *et al.* 2002). The carboxysomes in cyanelles presumably have the same function as those in bacteria. They certainly contain RUBISCO as do bacterial carboxysomes (Mangeney & Gibbs 1987; Mangeney *et al.* 1987). Molecular phylogenetic analysis indicates that the carboxysomes of cyanelles are β-carboxysomes whose functioning depends on the accumulation of a high concentration (*ca.* 10 mol m^{-3}) of inorganic carbon around the carboxysomes (Badger *et al.* 2002). Raven (1995) points out that the low osmolarity of freshwater cells whose volume regulation depends on active water efflux might restrict their metabolic activities, including the functioning of inorganic carbon concentrating mechanisms. The β-carboxysomal mechanism would be especially compromised in a low osmolarity compartment, and the retention of the peptido-

glycan wall by the cyanelles could facilitate functioning of the β-carboxysomes.

This possible functional relationship between the retention of the wall and of carboxysomes by cyanelles is consistent with the occurrence of carboxysomes in some marine dinoflagellates which are symbiotic in invertebrates (Blank 1986, 1987; Blank & Trench 1988). Here, the high osmolarity of the seawater means that any inorganic carbon accumulation required for carboxysome functioning in the dinoflagellate plastids could be readily accommodated in dinoflagellate cells with or without cell walls.

Other consequences of the presence of a peptidoglycan layer could include restrictions on the transfer of proteins produced in the cytosol by translation of nuclear-produced mRNA. However, this possibility does not seem to be realized (Schwartzbach *et al.* 1998; Steiner & Löffelhardt 2002).

The synthesis of a peptidoglycan wall as a means of volume regulation of a hyperosmolar cyanelle involves a one-time energy cost, whereas volume regulation for a wall-less hyperosmolar plastid via active water efflux is a continuing energy cost, with the relative costs of the two mechanisms depending on the growth rate of the cells (Raven 1982, 1995). Any competitive advantage gained by the (possible) higher osmolarity of cyanelles than of cytosol has clearly not been the route to great ecological success for glaucophytes as they are very minor components of the freshwater microflora today, in terms of both species number and species abundance. The genes known from the cyanelle genome of *Cyanophora* (Stinewalt *et al.* 1995; Löffelhardt *et al.* 1997*a*,*b*) do not give any clues as to osmoregulation or volume regulation of the cyanelles.

To summarize, there is overwhelming evidence that plastids have evolved from cyanobacterial ancestors but the crucial intermediate stages in the evolutionary transition from bacteria to bacterial-derived organelles are apparently unavailable. Perhaps, this evolutionary transition occurs very rarely (see § 4) or such genomes are transient because they are at a selective disadvantage relative to cyanobacteria and plastids. Recent studies on a very different group of associations, however, indicate that certain intracellular bacteria in insects may have the characteristics predicted of genomes at the interface between bacteria and bacterial-derived organelles. The second part of this article compares the characteristics of the genomes of these intracellular micro-organisms and bacterial-derived organelles, and the underlying evolutionary processes.

3. INTRACELLULAR MICRO-ORGANISMS IN INSECTS: GENOMES AT THE INTERFACE BETWEEN ORGANISMS AND ORGANELLES?

(a) *Microbial symbioses in insects*

Insect–microbial symbioses can be classified by the location of the micro-organisms: external to the insect (usually in the insect 'nests'), e.g. fungus-growing termites, leaf-cutting ants; in the insect gut, e.g. termites; and in specialized cells, known as mycetocytes or bacteriocytes. The 'mycetocyte symbioses' have evolved multiple times between various insect groups and a diverse array of micro-organisms. They include the cockroach–flavobacteria and the tsetse fly–*Wigglesworthia* (a γ-proteobacterium) symbioses, the associations between homopterans (aphids, whitefly, psyllids, planthoppers, etc.) and various bacteria and yeasts, and poorly studied microbial symbioses in sucking lice (Anoplura) and in various xylophagous beetles. Despite their independent evolutionary origins, these mycetocyte symbioses between insects and intracellular micro-organisms have two common characteristics. First, the microbial partner is obligately vertically transmitted, usually by insertion into the unfertilized eggs in the insect ovary. Second, the associations are mutually obligate, such that the micro-organisms cannot be brought into culture and are unknown apart from the insect hosts, and the insect, when experimentally deprived of their micro-organisms, grow slowly and are usually reproductively sterile. These micro-organisms are traditionally known as 'primary symbionts', as distinct from 'secondary symbionts', which are often (but not universally) found in various tissues of the insect hosts and are transmitted vertically with the primary symbionts. Further information about these symbioses can be obtained from the reviews of Buchner (1966), Baumann *et al.* (1995) and Douglas (1989, 1998*b*).

(b) *Microbial symbionts of insects as resident genomes*

A key characteristic shared by intracellular bacteria in various insects and bacterial-derived organelles is that they are 'resident genomes' *sensu* Andersson & Kurland (1998), i.e. they are obligately vertically transmitted from parent to offspring host with no opportunity for horizontal transmission. These resident genomes are, consequently, never exposed to the external environment or unrelated hosts. This has had a profound impact on the genomic evolution of the micro-organisms. In particular, it has promoted reduction in genome size. For example, the genome of *Wigglesworthia*, the symbiotic bacteria in tsetse flies is less than 0.77 Mbp (Akman & Aksoy 2001), and the genome of *Buchnera*, the symbiotic bacteria in aphids, is 0.45–0.64 Mbp, varying between aphid subfamilies (Shigenobu *et al.* 2000; Wernegreen *et al.* 2000; Gil *et al.* 2002). These values are intermediate between the genome sizes of related free-living bacteria and those of bacterial-derived organelles. *Wigglesworthia* and *Buchnera* are γ-Proteobacteria, allied to *Escherichia coli*, field isolates of which have genomes at 4.95 ± 0.25 Mbp (Bergthorsson & Ochman 1998). The genomes of mitochondria are generally 14–70 kilobase pairs (kbp) (although in plants can be as great as 2.6 Mbp) and plastids are *ca.* 120–200 kbp (table 3).

'Resident genomes' have two further characteristics: elevated rates of sequence evolution and AT bias (Andersson & Kurland 1998). For example, the substitution rates of 16S rRNA gene of *Buchnera* in aphids and *Carsonella* in psyllids have been estimated at 0.019–0.054 and 0.025–0.063 substitutions per site per 100 million years (Myr), up to nine times greater than the rates calculated for related enteric bacteria which are not intracellular (0.007–0.018 substitutions per site per 100 Myr) (Moran *et al.* 1993; Clark *et al.* 1999; Thao *et al.* 2000*a*). The GC content of the 16S rRNA gene of these three bacteria is 37% for *Carsonella*, 48% for *Buchnera* and 54% for *E. coli* K12 (calculated from GenBank accessions AF211151, NC002528, AE000129); and the average GC content of

the *Buchnera* and *E. coli* genomes is 26% and 51%, respectively (Blattner *et al.* 1997; Shigenobu *et al.* 2000).

These characteristics of small genome size, high AT content and rapid rates of sequence evolution are widely accepted to have common evolutionary bases: relaxed selection pressure and genomic decay through the accumulation of deleterious mutations (Moran 1996; Andersson & Kurland 1998; Moran & Wernegreen 2000). Any microbial traits that are either not required in the intracellular condition or are also present in the host are redundant and under relaxed selection. Even mutations that are slightly deleterious may accumulate by genetic drift because small numbers of micro-organisms are usually transmitted vertically, i.e. the microbial population is bottlenecked at each host generation. This leads to a genome-wide reduction in GC content because of the inherent mutational bias towards AT. The rate of genomic deterioration is elevated where genes for DNA recombination and repair are compromised; and the selection pressure on these genes may be relaxed because one important function, the elimination of invading DNA, may be redundant in the intracellular environment, which is sheltered from viruses, transposons etc. (Lawrence *et al.* 2001). The repeated loss of the most-fit genotypes in the resultant small, asexual populations of intracellular micro-organisms is referred to as Muller's ratchet (see § 2d(ii)).

The combined impacts of relaxed selection and Muller's ratchet on genomes of intracellular micro-organisms in insects can be illustrated by a non-bacterial example, a clade of ascomycete fungi informally known as yeast-like symbionts which occur in aphids of the tribe Cerataphini and certain planthoppers. In planthoppers, the yeast-like symbionts contribute to the insect nitrogen economy by degrading the insect excretory product, uric acid, via the enzyme uricase (Sasaki *et al.* 1996). Aphids, unlike planthoppers, do not produce uric acid, resulting in a predicted relaxation of selection pressure on the yeast-like symbiont uricase genes. Consistent with this expectation, the uricase genes in the yeast-like symbiont of aphids bear multiple mutations incompatible with uricase function (Hongoh & Ishikawa 2000). These include large deletions of the 5′-flanking region including the putative TATA box, frameshift mutations and, in one species examined, nonsense mutations (figure 1).

Sequence data are available for many genes of the γ-proteobacterium, *Buchnera*, the primary symbiont of virtually all aphids other than the tribe Cerataphini considered above. Across all the genes examined so far, the incidence of non-synonymous substitutions, K_a (i.e. point mutations that result in a change of amino acid), is elevated in *Buchnera* relative to the allied enteric bacteria *E. coli*/*Salmonella typhimurium*. As a consequence the ratio of synonymous/non-synonymous mutations (K_s/K_a) is consistently lower for *Buchnera* than for *E. coli*/*S. typhimurium* (figure 2). Compounded by the AT mutational bias, this results in a general difference in amino-acid composition of proteins between *Buchnera* and *E. coli*/*S. typhimurium*, to favour amino acids with codons of high AT content in *Buchnera* (e.g. asparagine, isoleucine, phenylalanine, tyrosine). Clark *et al.* (1999) argue that genomic decay arising from Muller's ratchet is a more plausible explanation than relaxed selection for the low K_s/K_a of *Buchnera* because the effects are evident across all loci studied,

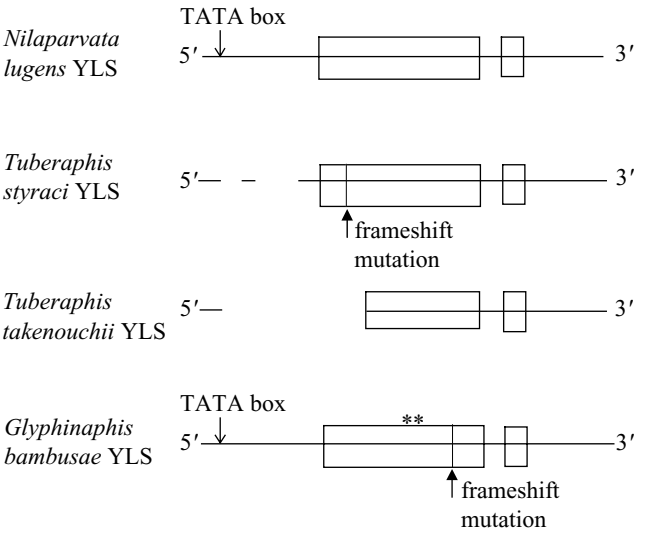

Figure 1. Deleterious mutations in the uricase gene of yeast-like symbiotic fungi (YLS) of aphids (*Tuberaphis* species and *Glyphinaphis bambusae*), as compared with the YLS of the planthopper *Nilaparvata lugens*. The exons are shown as open boxes, nonsense mutations as asterisks. *Tuberaphis* species additionally have deletions in the 5′ region, including the TATA box; and *T. styraci* and *G. bambusae* have frameshift mutations. (From fig. 6 of Hongoh & Ishikawa (2000).)

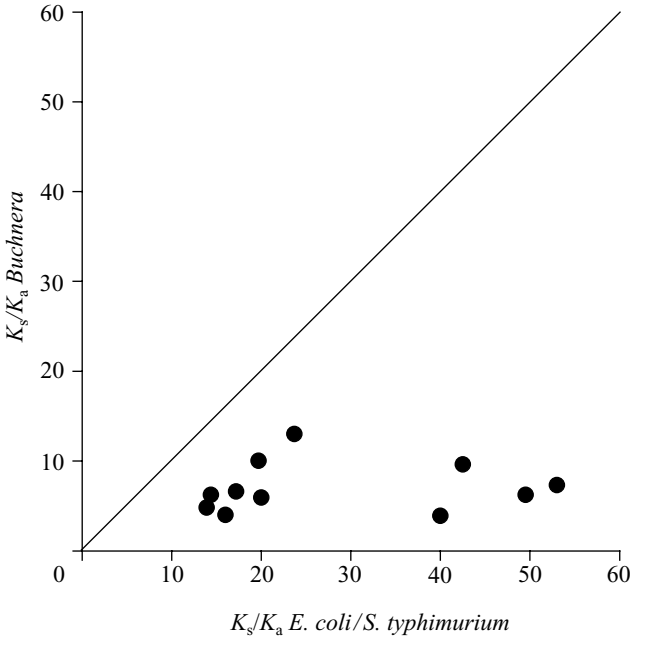

Figure 2. Ratio of synonymous/non-synonymous substitutions per site (K_s/K_a) for protein-coding genes between *Buchnera* in two aphid species (*Schizaphis graminum* and *Diuraphis noxia*) and between the enteric bacteria (*Escherichia coli* and *Salmonella typhimurium*) to which *Buchnera* is closely allied. (Redrawn from data in table 3 of Clark *et al.* (1999).)

including those related to the cooperative trait of amino-acid production (*ilv* and *trp* genes) and 'housekeeping' functions (e.g. *apt* genes and *ftsZ*). The high rates of sequence evolution in rRNA genes (see above) can also be attributed to genomic deterioration because many of

Table 4. Predicted stability of 16S rRNA of *Escherichia coli* and allied symbiotic γ-Proteobacteria in insects (data from Lambert & Moran (1998) and Spaulding & Von Dohlen (2001).)

taxon	$-\Delta G$ (kJ mole^{-1})	
	domain I of 16S rRNA	complete 16S rRNA
E. coli	−391	−2266
Buchnera (in aphids)	−272 to −316	−2052
'primary symbiont' of whitefly	−276	−1914
Carsonella (in psyllids)	not determined	−1463 to −1501

the substitutions have resulted in mismatches in stems or other paired regions of the RNA secondary structure and replacement of GC pairs by the less stable AU pairs. For 16S rRNA, the overall impact of these changes has been to reduce the energetic stability of the molecule, as indicated by the consistently less negative free energy ($-\Delta G$) for intracellular bacteria than *E. coli* (table 4) (Lambert & Moran 1998; Spaulding & Von Dohlen 2001).

(c) *Selection for function*

Although genomic decay is apparently general across genomes of intracellular symbionts, gene deletion is not random because of strong selection pressure for certain traits. Chief among these are cooperative traits, i.e. traits of selective advantage to the insect. If an insect benefits from a trait of its symbiotic bacteria, then a loss-of-function mutation contributing to that trait will depress the fitness of both the insect and, because they are obligately vertically transmitted, the symbiotic micro-organisms.

The key cooperative trait of bacteria in phloem-feeding insects (aphid, whitefly, psyllids, etc.) is the provision of essential amino acids, supplementing the poor supply of these nutrients in the diet (Douglas 1998*b*). Indicative of the predicted selection for this function, the gene inventory for the completely sequenced genome of *Buchnera* in the pea aphid *Acyrthosiphon pisum* includes 53 genes in essential amino-acid synthesis (*Buchnera* lacks orthologues for just three of the genes in this category present in the *E. coli* genome) but just 20–50% of the genes that, in *E. coli*, contribute to other biosynthetic functions, intermediate metabolism, DNA repair, etc. (Shigenobu *et al.* 2000). Blood-feeding insects (e.g. sucking lice, tsetse flies) are widely believed to derive B vitamins from their symbiotic bacteria (Buchner 1966). Although the genome of none of these bacteria has been sequenced, an estimated 85% of the ORFs of *Wigglesworthia*, the symbiont of tsetse flies, have been identified by array hybridization and, as predicted, this bacterium bears genes coding for key enzymes in B vitamin synthesis, e.g. *ribH* in riboflavin synthesis, *folA,C,D,K* in the synthesis of folate and dihydrofolate (Akman & Aksoy 2001).

In bacteria-derived organelles, selection for cooperative traits (oxygenic photosynthesis, aerobic respiration) is not equivalent to selection for retention of functional genes in the organelle genome because most of the genes coding for these traits are now borne in the nuclear genome. The translocation of DNA between genomes in eukaryotic cells occurs relatively readily and, once a functional copy of the gene is located in the nucleus, selection on its retention in an organelle subject to genomic decay is substantially relaxed. The net transfer to the nucleus of a gene whose product is targeted back to the organelle has been adopted as a defining feature (see § 1) of an organelle (Douglas 1994) and can be considered to both promote and compensate for genomic decay of the organelle.

Gene transfer to the nucleus and its impacts on genomic evolution of the bacteria/organelles is unlikely to be relevant to intracellular bacteria in insects because these bacteria are restricted to one specialized cell type, the mycetocyte, which does not give rise to germ cells. The most likely insect nucleus to receive bacterial DNA is the nucleus of the mycetocyte, which will die with the insect individual in which the DNA transfer occurred. This reasoning has been used as a general argument against the evolutionary origin of organelles in multicellular organisms with division between the germ line and soma (Douglas 1992; Kurland 2000). However, the possibility of bacterial-derived DNA, potentially including functional genes, in the insect nucleus cannot be ruled out because the vertically transmitted bacteria are localized to the cytoplasm of the unfertilized egg, in some insect groups for extended periods, providing an opportunity for DNA transfer from bacteria to germ line (Buchner 1966; Douglas 1989). To summarize, the implication of the localization of the bacteria to one differentiated cell type is that the bacterial genome is the prime focus of selection for function, although the incidence and extent of DNA transfer in these systems can only be resolved by extended sequence analysis of the insect genomes.

Symbiosis in multicellular hosts, such as insects, does however, offer an alternative route by which function can become independent of the retention of functional gene(s) in micro-organisms subject to Muller's ratchet. This arises from the presence of secondary symbionts in many insects (see above). The incidence of these vertically transmitted micro-organisms is variable. For example, all species of tsetse fly have the γ-proteobacterium *Sodalis*, although the abundance and tissue distribution varies with insect species (Chen & Aksoy 1999); different species of psyllids bear different taxa of secondary symbionts (Thao *et al.* 2000*b*), and some apparently have no secondary symbionts at all (Spaulding & Von Dohlen 2001); and five taxa of secondary symbionts have been identified in aphids, although the number and identity of taxa in aphids varies between and within species (e.g. Sandström *et al.* 2001). The limited information available indicates that the genomes of secondary symbionts are intermediate between free-living taxa and primary symbionts. For example, the genome size of *Sodalis* in tsetse flies, at 2 Mbp, lies between that of *E. coli* (more than 4 Mbp) and the tsetse primary symbiont, *Wigglesworthia* (less than 0.8 Mbp) (Akman *et al.* 2001), and the16S rRNA molecule of a secondary sym-

biont in psyllid has a higher calculated stability (-1881 kJ mole^{-1}) than the primary symbiont *Carsonella* (-1501 to -1463 kJ mole^{-1}) (Spaulding & Von Dohlen 2001). These data are consistent with the interpretation of various phylogenetic studies indicating that secondary symbionts have been acquired more recently than primary symbionts (e.g. Chen *et al.* 1999; Thao *et al.* 2000*b*; Darby *et al.* 2001; Sandström *et al.* 2001). The significance of secondary symbionts to the insect host is currently uncertain, but one possibility is that they act as 'helper' bacteria that supplement the cooperative traits of primary symbionts or support the metabolism of the primary symbionts (e.g. Gil *et al.* 2002). Such helper functions would reduce the selection on primary symbiont function. In other words, they may both enhance and compensate for the genomic deterioration of primary symbionts. We cannot exclude the possibility that the *Buchnera* in the ancestor of aphids of the tribe Cerataphini may have suffered total genomic meltdown through Muller's ratchet and been replaced by yeast-like symbionts.

Compensation for impaired function of symbionts by other members of the microbial community is more likely to occur in multicellular hosts than unicellular hosts, simply because the different taxa can be localized to different cells, and so coexist in the host. The competitive exclusion of symbiotic micro-organisms in unicellular hosts has been demonstrated by the studies of Görtz (1982) on *Paramecium* sp. This protist can bear stable populations of bacteria, algae or yeasts, but multiple infections do not persist because of a rigid competitive hierarchy with the algae and bacteria excluded by the yeasts, and bacteria also excluded by the algae. If the findings of Görtz (1982) are general, they could account for the sequential (not simultaneous) acquisition of bacteria and cyanobacteria and their transformation into mitochondria and plastids, respectively, as envisaged in the serial endosymbiosis theory of Margulis (1981).

These considerations indicate that the evolutionary fate of obligately vertically transmitted microbial symbionts may be strongly influenced by whether they are acquired by unicellular or multicellular hosts. Genomic deterioration in these symbionts is likely to be compensated for and promoted by net gene transfer to the nucleus of unicellular hosts (or hosts without separation of somatic cells from gametes), and by the transfer of function to secondary symbionts in multicellular hosts. However, we lack critical data on both secondary symbiont function and the incidence of microbial sequences in insect genomes, and therefore this distinction between unicellular and multicellular hosts should be seen as likelihood and not two mutually exclusive categories.

(d) *Functions beyond the genome of organelles and symbionts*

The functions of bacterial-derived organelles extend beyond the traits present in their bacterial ancestor and absent from their host. These organelles have acquired *in situ* additional functions as integrated parts of the eukaryotic cell, in both primary metabolism and signalling; for example, mitochondria are the site of fatty-acid oxidation, act as a Ca^{2+} store and are implicated in apoptosis (Berridge *et al.* 1998; Joza *et al.* 2001). Understanding of these functions is assisted by treating the organelles as membrane-bound compartments within the eukaryotic cell to which specific functions have been allocated, and not by referring to their ancestry as foreign organisms. These functions may have evolved as the control of the bacterial-derived organelles over their own metabolic pools and signalling networks declined, as a consequence of genomic deterioration. In other words, the supplementary functions of bacterial-derived organelles are the price they have paid for being rescued from genomic meltdown (i.e. total functional collapse) by the nucleocytoplasm.

To what extent does the insect nucleocytoplasm control the metabolic pools of their intracellular bacteria; and do these bacteria have generalized cellular functions independent of their roles in the synthesis of essential amino acids, vitamins, etc.? The genomic data indicate that *Buchnera* has very limited control over its own metabolism. In the *Buchnera* from the pea aphid, *Acyrthosiphon pisum*, all signalling systems, including two-component regulatory systems and quorum sensing are absent; transporter systems are restricted to a few ATP binding cassette transporters, and phosphotransferase systems for uptake of glucose and mannitol; and transcriptional controls over gene expression are apparently universally missing. Furthermore, *Buchnera* has no capacity for phospholipid synthesis, indicating that the bacteria derive either their total phospholipid requirement or phospholipid biosynthetic enzymes from the insect cytoplasm (Shigenobu *et al.* 2000). Such extensive loss of metabolic independence from the surrounding insect cell raises the possibility that these bacteria are integrated into insect cell function as a membrane-bound compartment. If such function(s) exist, however, then they are not general to all insect cells because the bacteria are restricted to mycetocytes. Furthermore, the affected organ systems would vary between insect taxa because the mycetocytes are, for example, borne in the fat body of cockroaches, haemocoele of aphids and the midgut epithelium of tsetse flies (Douglas 1989). In other words, the mycetocyte/bacteria complex may contribute to general fat body function in cockroaches, to the regulation of haemolymph composition in aphids, and to midgut function in tsetse flies. To our knowledge, such supplementary functions have not been investigated for any symbiosis, beyond the demonstration of Liadouze *et al.* (1995) that *Buchnera* contribute to the regulation of the haemolymph amino-acid composition of aphids.

In summary, the localization of intracellular bacteria to a single, differentiated cell type has restricted the opportunity for evolution of key traits of organelles, gene transfer to the nucleus and acquisition of supplementary functions as a membrane-bound subcellular compartment. Only further research will establish whether either or both of these traits have evolved in insect symbioses. If they have, then perhaps some of these systems may have crossed the evolutionary interface from bacterial symbionts to bacterial-derived organelles.

4. CONCLUSIONS

The single endosymbiotic event, which gave rise to all extant plastids, took place some two billion years ago. Could it be that this event (and the corresponding, rather earlier, unique event, which gave rise to all extant mito-

chondria and hydrogenosomes) was so unlikely that it only happened once? Because of the diversity of extant photosynthetic symbioses of cyanobacteria and non-photosynthetic eukaryotes it is unlikely that the initial stage of the symbiosis that gave rise to plastids was a unique event. It is likely that several associations would have been established between cyanobacteria and early mitochondria-containing non-photosynthetic eukaryotes. The progeny of any such symbioses no longer exist as resident genomes, with only the monophyletic plastids as resident genomes permitting photosynthesis in eukaryotes.

The absence of resident genomes permitting photosynthesis other than the plastids could be rationalized in at least two ways. One possible explanation is that several evolutionary lines of plastids became established about two billion years ago, and that all but one of them became extinct as a result of a global genetic bottleneck. Such a genetic bottleneck could have arisen in global glaciations in the Proterozoic aeon (Hoffman *et al.* 1998; Kirschvink *et al.* 2000), although the extent and biological impact of these glaciations is still a matter of debate (McKay 2000; Baum & Crowley 2001; James *et al.* 2001). The other possibility is that only the lineage of symbiotic cyanobacteria containing all extant plastids achieved the status of organelles, i.e. obligate symbionts with transfer of most of the genes needed for photosynthesis transferred to the host nucleus. The possible advantages of such a transfer include damage to the genome of photosynthetic organelles or proto-organelles owing to production of mutagenic active oxygen species in photosynthetic energy transformations. The genes remaining in the plastid genome include many whose expression is subject to transcriptional control by the plastid redox status. It could be that the first photosynthetic proto-organelle to reach organelle (plastid) status conferred such competitive advantage on the photosynthetic eukaryote with such organelles that any photosynthetic eukaryotes with photosynthetic proto-organelles became extinct. Such an argument could help to explain the absence of photosynthetic proto-organelles today in the lineage containing all extant plastids.

The occurrence of multiple, independently originating, endosymbiotic bacteria at the proto-organelle grade of evolution in phloem-feeding insects could be related to the problems of achieving organelle status for endosymbionts confined to a single, differentiated cell type in a multicellular organism.

REFERENCES

Akhmanova, A., Voncker, F., Van Alen, T., Van Hoek, A., Boxma, B., Vogels, G., Veenhuis, M. & Hackstein, J. H. P. 1998 A hydrogenosome with a genome. *Nature* **396**, 527–528.

Akman, L. & Aksoy, S. 2001 A novel application of gene arrays: *Escherichia coli* array provides insight into the biology of the obligate endosymbiont of tsetse flies. *Proc. Natl Acad. Sci. USA* **98**, 7546–7551.

Akman, L., Rio, R. V., Beard, C. B. & Aksoy, S. 2001 Genome size determination and coding capacity of *Sodalis glossinidius*, an enteric symbiont of tsetse flies, as revealed by hybridization to *Escherichia coli* gene arrays. *J. Bacteriol.* **183**, 4517–4525.

Allen, J. F. 1993*a* Redox control of gene expression and the function of chloroplast genomes: an hypothesis. *Photosynthesis Res.* **36**, 95–102.

Allen, J. F. 1993*b* Control of gene expression by redox potential and the requirement for chloroplast and mitochondrial genomes. *J. Theor. Biol.* **165**, 609–631.

Allen, J. F. 1993*c* Redox control of transcription: sensors, responses, regulators, activators and repressors. *FEBS Lett.* **332**, 203–207.

Allen, J. F. 1995 Thylakoid protein phosphorylation, state 1–state 2 transition, and photosystem stoichiometry adjustment: redox control at multiple levels of gene expression. *Physiol. Plant* **93**, 196–205.

Allen, J. F. 1996 Separate sexes and the mitochondrial theory of ageing. *J. Theor. Biol.* **180**, 135–140.

Allen, J. F. & Nilsson, A. 1997 Redox signalling and the structural basis of photosynthesis by protein phosphorylation. *Physiol. Plant* **100**, 863–868.

Allen, J. F. & Raven, J. A. 1996 Free-radical-induced mutation vs redox regulation: costs and benefits of genes in organelles. *J. Mol. Evol.* **42**, 482–492.

Andersson, S. G. E. & Kurland, C. G. 1998 Reductive evolution of resident genomes. *Trends Microbiol.* **6**, 263–268.

Badger, M. R., Hanson, D. & Price, G. D. 2002 Evolution and diversity of CO_2 concentrating mechanisms in cyanobacteria. *Funct. Plant Biol.* **29**, 161–173.

Barbrook, A. C., Symington, H., Nisbet, R. E. R., Larkum, A. & Howe, C. J. 2001 Organisation and expression of the plastid genome of the dinoflagellate *Amphidinium operculatum*. *Mol. Genet. Genomics* **266**, 623–638.

Baum, S. K. & Crowley, T. J. 2001 GCM response to late Pre-Cambrian (similar to 590 Ma) ice-covered continents. *Geophys. Res. Lett.* **28**, 583–586.

Baumann, P., Baumann, L., Lai, C. Y., Roubakhsh, D., Moran, N. A. & Clark, M. A. 1995 Genetics, physiology, and evolutionary relationships of the genus *Buchnera*: intracellular symbionts of aphids. *A. Rev. Microbiol.* **49**, 55–94.

Beech, P. L. & Gilson, P. R. 2000 Fts and organelle division in protists. *Protist* **151**, 11–16.

Bergthorsson, U. & Ochman, H. 1998 Distribution of chromosome length variation in natural isolates of *Escherichia coli*. *Mol. Biol. Evol.* **15**, 6–16.

Berridge, M. J., Bootman, M. D. & Lipp, P. 1998 Calcium: a life and death signal. *Nature* **395**, 645–648.

Bhattacharya, D. T., Helmchen, T., Bibeau, C. & Melkonian, M. 1995 Comparisons of nuclear-encoded small-subunit ribosomal RNAs reveal the evolutionary position of the Glaucocystophytes. *Mol. Biol. Evol.* **12**, 415–420.

Blanchard, J. L. & Lynch, M. 2000 Organellar genes. Why do they end up in the nucleus? *Trends Genet.* **16**, 315–320.

Blank, R. J. 1986 Unusual chloroplast structure in endosymbiotic dinoflagellates: a clue to evolutionary differentiation within the genus *Symbiodinium* (Dinophyceae). *Plant Syst. Evol.* **151**, 271–280.

Blank, R. J. 1987 Cell architecture of the dinoflagellate *Symbiodinium* sp. inhabiting the Hawaiian stony coral *Montipora verrucosa*. *Mar. Biol* **94**, 143–155.

Blank, R. J. & Trench, R. K. 1988 Immunogold localization of ribulose-1,5-bisphosphate carboxylase-oxygenase in *Symbiodinium kawaguti* Trench and Black, an endosymbiotic dinoflagellate. *Endocytobiol. Cell Res.* **5**, 75–82.

Blattner, F. R. (and 16 others) 1997 The complete genome sequence of *Escherichia coli* K12. *Science* **277**, 1453–1462.

Buchner, P. 1966 *Endosymbiosis of animals with plant microorganisms*. London: Wiley.

Chen, X. & Aksoy, S. 1999 Tissue tropism, transmission and expression of foreign genes *in vivo* in midgut symbionts of tsetse flies. *Insect Mol. Biol.* **8**, 125–132.

Chen, X., Li, S. & Aksoy, S. 1999 Concordant evolution of a symbiont with its host insect species: molecular phylogeny of the genus *Glossinia* and its bacteriome-associated endosymbiont, *Wigglesworthia glossinida*. *J. Mol. Evol.* **48**, 49–58.

Clark, M. A., Moran, N. A. & Baumann, P. 1999 Sequence evolution in bacterial endosymbionts having extreme base compositions. *Mol. Biol. Evol.* **16**, 1586–1598.

Clauss, H., Martin, H. H., Jantes, C. A. & Konig, H. 2000 A search for β-lactamase in chlamydiae, mycoplasmas, planktomycetes and cyanelles: bacteria and bacterial descendants at different phylogenetic positions and stages of cell wall development. *Microb. Res.* **155**, 1–6.

Darby, A. C., Birkle, L. M., Turner, S. L. & Douglas, A. E. 2001 An aphid-borne bacterium allied to the secondary symbionts of whitefly. *FEMS Microbiol. Ecol.* **36**, 43–50.

Douglas, A. E. 1989 Mycetocyte symbiosis in insects. *Biol. Rev.* **69**, 409–434.

Douglas, A. E. 1992 Symbiosis in evolution. *Oxf. Surv. Evol. Biol.* **8**, 347–382.

Douglas, A. E. 1994 *Symbiotic interactions*. Oxford University Press.

Douglas, A. E. 1998*a* Host benefit and the evolution of specialization in symbiosis. *Heredity* **81**, 599–603.

Douglas, A. E. 1998*b* Nutritional interactions in insect–microbial symbioses. *A. Rev. Entomol.* **43**, 17–37.

Douglas, S. E. & Penny, S. L. 1999 The plastid genome of the cryptophyte alga, *Guillardia theta*: complete sequence and conserved synteny groups confirm its common ancestry with red algae. *J. Mol. Evol.* **48**, 236–244.

Ferriere, R., Bronstein, J. L., Rinaldi, S., Law, R. & Gauduchon, M. 2002 Cheating and evolutionary stability in mutualisms. *Proc. R. Soc. Lond.* B **269**, 773–780. (DOI 10.1098/rspb.2001.1900.)

Finlay, B. J., Span, A. S. W. & Harman, J. M. P. 1983 Nitrate respiration in primitive eukaryotes. *Nature* **303**, 333–336.

Galvan, A., Rexach, J., Mariçal, V. & Fernandez, E. 2002 Nitrite transport to the chloroplast in *Chlamydomonas reinhardtii*: molecular evidence for a regulated process. *J. Exp. Bot.* **53**, 845–853.

Gil, R., Sabater-Munoz, B., Latorre, A., Silva, F. J. & Moya, A. 2002 Extreme genome reduction in *Buchnera* spp; toward the minimal genome needed for symbiotic life. *Proc. Natl Acad. Sci. USA* **99**, 4454–4458.

Glockner, G., Rosenthal, A. & Valentin, K. 2000 The structure and gene content of an ancient red algal genome. *J. Mol. Evol.* **51**, 382–399.

Görtz, H. D. 1982 Infections of *Paramecium bursaria* with bacteria and yeasts. *J. Cell Sci.* **58**, 445–453.

Grant, B. R. & Borowitzka, M. A. 1984*a* The chloroplasts of giant-celled and coenocytic algae: biochemistry and structure. *Bot. Rev.* **50**, 267–307.

Grant, B. R. & Borowitzka, M. A. 1984*b* Changes in structure of isolated chloroplasts of *Codium fragile* and *Caulerpa filiformis* in response to osmotic shock and detergent treatment. *Protoplasma* **120**, 155–164.

Green, B. J., Li, W.-Y., Manhart, J. R., Fox, T. C., Summer, E. J., Kennedy, R. A., Pierce, S. K. & Rumpho, M. E. 2000 Mollusc–alga chloroplast endosymbiosis. Photosynthesis, thylakoid protein maintenance, and chloroplast gene expression continue for many months in the absence of the algal nucleus. *Plant Physiol.* **124**, 331–342.

Hackstein, J. H. P., Akhmanova, A., Van Hoek, A., Van Alen, T., Boxma, B., Moon-van der Staay, S. Y., Van der Staay, G., Leunisson, M., Rosenberg, S. & Veenhuis, M. 2001 Hydrogenosomes: convergent adaptations of mitochondria to anaerobic environments. *Zool. Anal. Compl. Syst.* **104**, 290–302.

Harten, J. J. & Pierce, S. K. 2001 Synthesis of several light-harvesting complex I polypeptides is blocked by cycloheximide in the sea slug *Elysia chlorotica* (Gould): a case for horizontal gene transfer between alga and animal. *Biol. Bull.* **201**, 34–44.

Helmchen, T. A., Bhattacharya, D. & Melkonian, M. 1995 Analyses of ribosomal-RNA sequences from glaucocystophyte cyanelles provide new insights into the evolutionary relationships of plastids. *J. Mol. Evol.* **41**, 203–210.

Henze, K. & Martin, W. 2001 How do mitochondrial genes get into the nucleus? *Trends Gen.* **17**, 383–387.

Hess, W. R., Rocap, G., Ting, C. S., Larimer, F., Stilwagen, S., Lamendin, J. & Chisholm, S. W. 2001 The photosynthetic apparatus of *Prochlorococcus*: insights through comparative genomics. *Photosynthesis Res.* **70**, 53–71.

Hoffman, P. F., Kaufman, A. J., Halverson, G. P. & Schrag, D. P. 1998 A Neoproterozoic snowball earth. *Science* **281**, 1342–1346.

Hongoh, Y. & Ishikawa, H. 2000 Evolutionary studies on uricases of fungal endosymbionts of aphids and planthoppers. *J. Mol. Evol.* **51**, 265–277.

Howe, C. J., Barbrook, A. C. & Lockhart, P. J. 2000 Organelle genes: do they jump or are they pushed? *Trends Genet.* **16**, 65–66.

James, N. P., Narbonne, G. M. & Keyser, T. K. 2001 Late Neoproterozoic cap carbonates: Mackenzie Mountains, Northwest Canada: precipitation and global glacial meltdown. *Can. J. Earth Sci.* **38**, 1229–1262.

Joza, N. (and 19 others) 2001 Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. *Nature* **410**, 549–554.

Kaneko, T. (and 23 others) 1996 Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res.* **3**, 188–201.

Kanerski, I. & Maliga, P. 1994 Relocation of the plastid *rbcL* gene to the nucleus yields functional ribulose-1,5-bisphosphate carboxylase. *Proc. Natl Acad. Sci. USA* **91**, 1969–1973.

Kasten, B. & Reski, R. 1997 β-lactam antibiotics inhibit chloroplast division in a moss (*Physcomitrella patens*) but not in tomato (*Lycopersicon esculentum*). *J. Plant Physiol.* **150**, 137–140.

Kies, L. 1992 Glaucocystophyceae and other protists harbouring prokaryotic endocytobionts. In *Algae and symbioses* (ed. W. Reisser), pp. 353–377. Bristol, UK: Biopress Ltd.

Kirschvink, J. L., Gaidos, E. J., Bertanik, L. E., Beukes, N. J., Gutzmer, J., Maepa, L. N. & Steinberger, R. E. 2000 Palaeoproterozoic snowball Earth. *Proc. Natl Acad. Sci. USA* **97**, 1400–1405.

Koch, A. L. 2001 *Bacterial growth and form*. Dordrecht, The Netherlands: Kluwer.

Kowallik, K. V., Stoebe, B., Schaffran, I., Krothparic, P. & Frier, U. 1995 The chloroplast genome of a chlorophyll *a* + *c* containing alga *Odontella sinensis*. *Plant Mol. Biol. Reporter* **13**, 336–342.

Kurland, C. G. 2000 Something for everyone: horizontal gene transfer in evolution. *EMBO Rep.* **1**, 1–4.

Lambert, J. D. & Moran, N. A. 1998 Deleterious mutations destabilize ribosomal RNA in endosymbiotic bacteria. *Proc. Natl Acad. Sci. USA* **95**, 4458–4462.

Larkum, A. W. D. 1968 Ionic relations of chloroplasts *in vivo*. *Nature* **218**, 447–450.

Lawrence, J. M., Hendrix, R. W. & Casjens, S. 2001 Where are the pseudogenes in bacterial genomes? *Trends Microbiol.* **9**, 535–540.

Lemieux, C., Otis, C. & Turmel, M. 2000 Ancestral chloroplast genome in *Mesostigma viride* reveals an early branch of green plant evolution. *Nature* **403**, 649–652.

Liadouze, I., Febvay, G., Guillaud, J. & Bonnot, G. 1995 Effect of diet on the free amino acid pools of symbiotic and aposymbiotic pea aphids, *Acyrthosiphon pisum*. *J. Insect Physiol.* **41**, 33–40.

Liaimer, A. 2002 Molecular communication and responses in *Nostoc*-plant symbioses. PhD thesis, University of Stockholm, Sweden.

Lockhart, P. J., Howe, C. J., Bryant, D. A., Beanland, T. J. & Larkum, A. W. D. 1992 Substitutional bias confounds inference of cyanelle origins from sequence data. *J. Mol. Evol.* **34**, 153–162.

Löffelhardt, W., Bohnert, H. J. & Bryant, D. A. 1997*a* The complete sequence of the *Cyanophora paradoxa* cyanelle genome (Glaucocystophyceae). *Plant Syst. Evol. Suppl.* **11**, 149–162.

Löffelhardt, W., Bohnert, H. J. & Bryant, D. A. 1997*b* The cyanelles of *Cyanophora paradoxa*. *Crit. Rev. Plant Sci* **16**, 393–413.

McFadden, G. I. 1999 Endosymbiosis and evolution in the plant cell. *Curr. Opin. Plant Biol.* **2**, 513–519.

McFadden, G. I. 2001 Primary and secondary endosymbiosis and the origin of plastids. *J. Phycol.* **37**, 951–959.

McKay, C. P. 2000 Thickness of tropical ice and photosynthesis on a snowball Earth. *Geophys. Res. Lett.* **27**, 2153–2156.

Mallick, N., Rai, L. C., Mohn, F. H. & Soeder, C. J. 1999 Studies on nitric oxide (NO) formation by the green alga *Scenedesmus obliquus* and the diazotrophic cyanobacterium, *Anabaena doliolum*. *Chemosphere* **39**, 1601–1610.

Mallick, N., Mohn, F. H., Rai, L. C. & Soeder, C. J. 2000*a* Evidence for the non-involvement of nitrite reductase in nitric oxide production by the green alga *Scenedesmus obliquus*. *J. Plant Physiol.* **156**, 423–426.

Mallick, N., Mohn, F. H., Rai, L. & Soeder, C. J. 2000*b* Impact of physiological stresses on nitric oxide formation by green alga, *Scenedesmus obliquus*. *J. Microbiol. Biotech.* **10**, 300–306.

Mallick, N., Mohn, F. H. & Soeder, C. J. 2000*c* Evidence supporting nitrite-dependent NO release by the green microalga *Scenedesmus obliquus*. *J. Plant Physiol.* **157**, 40–46.

Mangeney, E. & Gibbs, S. P. 1987 Immunocytochemical localization of ribulose-1,5-bisphosphate carboxylase oxygenase in the cyanelles of *Cyanophora paradoxa* and *Glaucocystis nostochinearum*. *Eur. J. Cell Biol.* **43**, 65–70.

Mangeney, E., Hawthornthwaite, A. M., Codd, G. A. & Gibbs, S. P. 1987 Immunocytochemical localization of phosphoribulose kinase in the cyanelles of *Cyanophora paradoxa* and *Glaucocystis nostochinearum*. *Plant Physiol.* **84**, 1028–1032.

Margulis, L. 1981 *Symbiosis in cell evolution*. San Fransisco, CA: Freeman & Co.

Martin, W. & Kowallik, K. 1999 Annotated English translation of Mereschowsky's 1905 paper 'Uber Natur und Ursprung der Chromatophoren in Pflanzenreiche'. *Eur. J. Phycol.* **34**, 287–295.

Martin, W., Stoebe, B., Goremykin, V., Hasegawa, M. & Kowallik, K. V. 1998 Gene transfer to the nucleus and the evolution of chloroplasts. *Nature* **393**, 162–165.

Martin, W., Rujan, T., Richly, E., Hansen, A., Cornelson, S., Lins, T., Leister, D., Stoebe, B., Hasegawa, M. & Penny, D. 2002 Evolutionary analysis of *Arabidopsis,* cyanobacterial and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. *Proc. Natl Acad. Sci. USA* **99**, 12 246–12 251.

Meeks, J. C., Elhai, J., Thiel, T., Potts, M., Larimer, F., Lamerdin, J., Predki, P. & Atlas, R. 2001 An overview of the genome of *Nostoc punctiforme*, a multicellular, symbiotic, cyanobacterium. *Photosynthesis Res.* **70**, 85–106.

Mereschowsky, C. 1905 Uber Natur und Ursprung der Chromatophoren in Pflanzenreich. *Biol. Centralbl.* **25**, 593–604; 689–691.

Miller, R. S. (and 12 others) 2001 Many parallel losses of infA from chloroplast DNA during angiosperm evolution with multiple independent transfers to the nucleus. *Plant Cell* **13**, 645–658.

Moran, N. A. 1996 Accelerated evolution and Muller's rachet in endosymbiotic bacteria. *Proc. Natl Acad. Sci. USA* **93**, 2873–2878.

Moran, N. A. & Wernegreen, J. J. 2000 Lifestyle evolution in symbiotic bacteria: insights from genomics. *Trends Ecol. Evol.* **15**, 321–326.

Moran, N. A., Munson, M. A., Baumann, P. & Ishikawa, H. 1993 A molecular clock in endosymbiotic bacteria is calibrated using the insect hosts. *Proc. R. Soc. Lond.* B **253**, 167–171.

Moreira, D., Le Guyader, H. & Philippe, H. 2000 The origin of red algae and the evolution of chloroplasts. *Nature* **405**, 69–72.

Morse, D., Salois, P., Markovic, P. & Hastings, J. W. 1995 A nuclear-encoded form II RUBISCO in dinoflagellates. *Science* **268**, 1622–1624.

Morton, B. R. 1998 Selection of the codon bias of chloroplast and cyanelle genes in different plant and algal lineages. *J. Mol. Evol.* **46**, 449–459.

Morton, B. R. 2001 Selection at the amino acid level can influence synonymous codon usage: implications for the study of codon adaptation in plastid genes. *Genetics* **159**, 347–358.

Nobel, P. S. 1975 Chloroplasts. In *Ion transport in plant cell and tissues* (ed. D. A. Baker & J. L. Hall), pp. 101–124. Amsterdam: North Holland/American Elsevier.

Palenik, B. 2002 The genomes of symbiosis: hosts keep the baby and the bath water. *Proc. Natl Acad. Sci. USA* **99**, 11 996–11 997.

Pfannschmidt, T., Nilsson, A. & Allen, J. F. 1999 Photosynthesis control of chloroplast gene expression. *Nature* **397**, 625–628.

Pfannschmidt, T., Allen, J. F. & Oelmuller, R. 2001*a* Principles of redox control in photosynthesis gene expression. *Physiol. Plant* **112**, 1–9.

Pfannschmidt, T., Schultze, K., Brost, M. & Oelmuller, R. 2001*b* A novel mechanism of nuclear photosynthesis gene regulation by redox signals from the chloroplast during photosystem stoichiometry adjustment. *J. Biol. Chem.* **276**, 36 125–36 130.

Pfanzagl, B., Zenker, A., Pittenauer, E., Allmaier, G., Martinez Torrecuddna, J., Schmid, E. R., De Pedro, M. A. & Löffelhardt, W. 1996 Primary structure of cyanelle peptidoglycan of *Cyanophora paradoxa*: a prokaryotic cell wall as part of an organelle envelope. *J. Bacteriol.* **178**, 332–339.

Race, H. L., Hermann, R. G. & Martin, W. 1999 Why have organelles retained genomes? *Trends Genet.* **15**, 364–370.

Rai, A. N., Söderback, E. & Bergman, B. 2000 Cyanobacterium–plant symbioses. *New Phytol.* **147**, 449–481.

Raven, J. A. 1982 The energetics of freshwater algae: energy requirements for biosynthesis and volume regulation. *New Phytol.* **92**, 1–20.

Raven, J. A. 1984 *Energetics and transport in aquatic plants*. New York: A. R. Liss.

Raven, J. A. 1995 Costs and benefits of low intracellular osmolarity in cells of freshwater algae. *Funct. Ecol.* **9**, 701–707.

Raven, J. A. 2002*a* The evolution of cyanobacterial symbioses. *Biol. Environ. Proc. R. Irish Acad.* **102B**, 3–6.

Raven, J. A. 2002*b* Evolution of cyanobacterial symbioses. In *Cyanobacteria in symbiosis* (ed. A. N. Rai, B. Bergman & U. Rasmussen), pp. 239–333. Dordrecht, The Netherlands: Kluwer.

Raven, J. A. 2003 Carboxysomes and peptidoglycan walls of cyanelles: possible physiological functions. *Eur. J. Physiol.* **38**. (In the press.)

Raven, J. A., Walker, D. I., Jensen, K. R., Handley, L. L., Scrimgeour, C. M. & McInroy, S. G. 2001 What fraction of the organic carbon sacoglossans is obtained from photosynthesis by kleptoplastids? An investigation using the natural abundance of stable carbon isotopes. *Mar. Biol.* **138**, 537–545.

Reith, M. E. 1995 Molecular biology of rhodophyte and chromophyte plastids. *A. Rev. Plant Physiol. Plant Mol. Biol.* **46**, 549–575.

Reith, M. E. & Mulholland, J. 1995 Complete nucleotide sequence of the *Porphyra purpurea* chloroplast genome. *Plant Mol. Biol. Rep.* **13**, 333–335.

Rippka, R., Dervelles, J., Waterbury, J. B., Herdman, M. & Stariner, R. Y. 1979 Genetic assignments, strain histories and properties of pure cultures of cyanobacteria. *J. Gen. Microbiol.* **111**, 1–61.

Rujan, T. & Martin, W. 2001 How many genes in *Arabidopsis* come from cyanobacteria. An estimate from 386 protein phylogenies. *Trends Genet.* **17**, 113–120.

Rumpho, M. E., Summer, E. J. & Manhart, J. R. 2000 Solar-powered sea slugs. Mollusc/algal chloroplast symbioses. *Plant Physiol.* **123**, 29–38.

Saccone, C., Gissi, C., Lanove, C., Larizza, A., Pesole, G. & Reyes, A. 2000 Evolution of the mitochondrial genetic system: an overview. *Gene* **261**, 153–159.

Sandström, J. P., Russell, J. A., White, J. P. & Moran, N. A. 2001 Independent origins and horizontal transfer of bacterial symbionts of aphids. *Mol. Ecol.* **10**, 217–228.

Sasaki, T., Kawamura, M. & Ishikawa, H. 1996 Nitrogen recycling in the brown planthopper, *Nilaparvata lugens*: involvement of yeast-like endosymbionts in uric acid metabolism. *J. Insect Physiol.* **42**, 125–129.

Schwartzbach, S. D., Osafune, T. & Löffelhardt, W. 1998 Protein import into cyanelles and complex chloroplasts. *Plant Mol. Biol.* **38**, 247–263.

Schyns, G., Rippka, R., Namawe, A., Campbell, D., Herdman, M. & Houmard, J. 1997 *Prochlorothrix hollandica* PCC 9006: genomic properties of an axenic representative of the chlorophyll *a*/*b* containing oxyphotobacteria. *Res. Microbiol.* **148**, 348–354.

Selosse, M. A., Albert, B. R. & Godelle, B. 2001 Reducing the genome size in organelles favours gene transfer to the nucleus. *Trends Ecol. Evol.* **16**, 135–141.

Shigenobu, S., Watanabe, H., Hattori, M., Sakaki, Y. & Ishikawa, H. 2000 Genome sequence of the endocellular bacterial symbiont of aphids *Buchnera* sp. APS. *Nature* **407**, 81–86.

Smith, D. C. & Douglas, A. E. 1987 *The biology of symbiosis*. London: Edward Arnold.

Spaulding, A. W. & Von Dohlen, C. D. 2001 Psyllid endosymbionts exhibit patterns of cospeciation with hosts and destabilizing substitutions in ribosomal RNA. *Insect Mol. Biol.* **10**, 57–67.

Steiner, J. M. & Löffelhardt, W. 2002 Protein import into cyanelles. *Trends Plant Sci.* **7**, 72–77.

Stiller, J. W. & Hall, D. B. 1997 The origin of red algae: implications for plastid evolution. *Proc. Natl Acad. Sci. USA* **94**, 4220–4225.

Stiller, J. W. & Hall, D. B. 1998 Sequences of the largest subunits of RNA polymerase II from two red algae and their implications for rhodophyte evolution. *J. Phycol.* **34**, 857–864.

Stiller, J. W., Riley, J. & Hall, D. B. 2001 Are red algae plants? A critical evaluation of three key molecular data sets. *J. Mol. Evol.* **52**, 527–539.

Stinewalt, V. L., Michalowski, C. B., Löffelhardt, W., Bohnert, H. J. & Bryant, D. A. 1995 Nucleotide sequence of the cyanelle genome from *Cyanophora paradoxa*. *Plant Mol. Biol. Rep.* **13**, 327–332.

Stoebe, B. & Kowallik, K. V. 1999 Gene-cluster analysis in chloroplast genomics. *Trends Genet.* **15**, 344–347.

Surpin, M., Larkin, R. M. & Chory, J. 2002 Signal transduction between the chloroplast and the nucleus. *Plant Cell* **14**(Suppl. 1), S327–S338.

Thao, M. L., Moran, N. A., Abbott, P., Brennan, E. B., Burckhardt, D. H. & Baumann, P. 2000*a* Cospeciation of psyllids and their primary prokaryotic endosymbionts. *Appl. Environ. Microbiol.* **66**, 2898–2905.

Thao, M. L., Clark, M. A., Baumann, L., Brennan, E. B., Moran, N. A. & Baumann, P. 2000*b* Secondary endosymbionts of psyllids have been acquired multiple times. *Curr. Microbiol.* **41**, 300–304.

Ting, C. S., Rocap, G., King, J. & Chisholm, S. W. 2002 Cyanobacterial photosynthesis in the oceans: the origin and significance of divergent light-harvesting strategies. *Trends Microbiol.* **10**, 134–142.

Tomitani, A., Okaca, K., Miyashita, H., Matthijs, H. C. P., Ohno, T. & Tanaka, A. 1999 Chlorophyll *b* and phycobilins in the common ancestor of cyanobacteria and chloroplasts. *Nature* **400**, 159–162.

Turmel, M., Otis, C. & Lemieux, C. 1999 The complete chloroplast DNA sequence of the green alga *Nephroselmis olivacea*. *Proc. Natl Acad. Sci. USA* **96**, 10 248–10 253.

Turner, S., Pryer, K. M., Miao, V. P. W. & Palmer, J. D. 1999 Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. *J. Eukaryot. Microbiol.* **46**, 327–338.

Van den Hoek, C., Mann, D. G. & Jahns, H. M. 1995 *Algae. An introduction to phycology*. Cambridge University Press.

Walsby, A. E. 1980 The water relations of gas-vacuolate prokaryotes. *Proc. R. Soc. Lond.* B **208**, 73–102.

Walsby, A. E. 1986 The pressure relationships of halophilic and non-halophilic prokaryotic cells determined by using gas vesicles as pressure probes. *FEMS Rev.* **39**, 45–49.

Wernegreen, J. J., Ochman, H., Jones, I. B. & Moran, N. A. 2000 Decoupling of genome size and sequence divergence in a symbiotic bacterium. *J. Bacteriol.* **182**, 3867–3869.

Whitney, S. M. & Yellowlees, D. 1995 Preliminary investigations into the structure and activity of ribulose bisphosphate carboxylase from two photosynthetic dinoflagellates. *J. Phycol.* **31**, 138–146.

Whitney, S. M., Shaw, D. C. & Yellowlees, D. 1995 Evidence that some dinoflagellates contain a ribulose 1,5-bisphosphate carboxylase/oxygenase related to that of α-proteobacteria. *Phil. Trans. R. Soc. Lond.* B **259**, 271–275.

Zerges, W. 2000 Translation in chloroplasts. *Biochimie* **82**, 583–601.

Zerges, W. 2002 Does complexity constrain organelle evolution? *Trends Plant Sci.* **7**, 175–182.

Discussion

D. Horner (*Dipartimento di Fisiologia e Biochimica Generali, University of Milan, Milan, Italy*). Is there significant heteroplasmy in the *Buchnera* population? Are there significant variants within a single organism in the genome?

A. E. Douglas. In our experience the answer is 'no'. Our population is very uniform, just what you would expect for a very small effective population size.

D. Horner. I wanted just to make a comment about the first part of the talk but I am not completely sure that citing hydrogenosome genome loss as being consistent with some of the models of retention and loss of organelle genomes is a particularly good argument because I am not aware of any published cases where any of the core mitochondrial genes have been described in hydrogenosome-containing organisms. So, it is not a case of the pressure to keep them on the genome having changed and them having moved to the nucleus. However, we do not have the complete genomes yet.

J. A. Raven. Absolutely. I was perhaps trying to make the general case rather than be specific for any gene, which

really means that my case fails if I cannot specify a gene that is or is not regulated by the redox state of the hydrogenosome.

T. Cavalier-Smith (*Department of Zoology, University of Oxford, Oxford, UK*). I am sceptical of the frequent invocation of Muller's ratchet in relation to organelles, especially mitochondria and chloroplasts, but also, I think, these insect symbionts. The fundamental force in Muller's ratchet is random drift, which depends on small populations, but I do not think the small populations within the cell or within the organism are really relevant. With an alga or a free-living protozoan the populations are in billions of billions so random drift is not going to be a very important factor in the spread of less adaptive combinations. Therefore, I do not think that this is the primary consideration in these cases. I think that the same would apply to most insect populations. There are a lot of aphids around.

A. E. Douglas (*Department of Biology, University of York, York, UK*). Yes, but the effective population size for the microbial partner is the number of bacterial cells that are transmitted to each of the subsequent generations.

T. Cavalier-Smith. It depends what you mean by 'effective'. If in one lineage you do lose the most highly adaptive combination, another aphid somewhere else, maybe in the same field, would have it. If the first one is less efficient in some respect, selection could act between them and so it would not spread through the whole population. Therefore, I do not think it is fundamentally different from mutation.

A. E. Douglas. We would expect Muller's ratchet to be countered by selection at the level of the intact symbiosis. Yes, I would agree with that.

W. Martin (*Institute of Botany III, Heinrich-Heine Universität, Düsseldorf, Düsseldorf, Germany*). Two very brief comments and a question. First, I would like to disagree with David Horner concerning the hydrogenosome argument as not being consistent with John Allen's model for the retention of genomes, because, if you recall, there are two kinds of anaerobic mitochondria: those that have genomes and those that do not. We will hear more about anaerobic mitochondria from Louis Tielens. The mitochondria that have retained membrane-associated electron transport have retained the genome, and those that have lost membrane-associated electron transport have lost it, so keep that in mind.

W. Martin. The second comment is a little longer. You mentioned the Von Dohlen group, which found evidence, published last year in *Nature*, for gamma proteobacterial symbionts within beta proteobacterial hosts in the mealy bug association. I find that to be particularly important because when we think about the origins of mitochondria there is a century-old notion that the host that acquired the mitochondrion had to be a eukaryote because of the mechanism for entry. What Von Dohlen's finding shows is that the host did not necessarily have to be a eukaryote because it gives us an example of a eubacterial symbiont within a prokaryotic host. My question is, then, do we know of other examples where prokaryotic endosymbionts live within prokaryotic hosts?

A. E. Douglas. I am not aware of other examples and the situation described by Von Dohlen in that particular set-up actually relates to a very small number of cilids. Not all cilids have them, just a few. It is a truly extraordinary situation that she has identified, and I suspect it is very rare.

R. G. Herrmann (*Department für Biologie I, Bereich Botanik, Ludwig-Maximilians-Universität, Munich, Germany*). A comment on the reason that John Raven gave for gene retention. The first point you made was that probably some large proteins cannot be imported, and in practice you ruled that this was unlikely. Well I can tell you that even proteins with 11 trans-membrane spans can be successfully imported. What is difficult is to get is full functionality of the protein in the compartment, even with the action of chaperones. A post-translational import does not always guarantee that the chaperone interacts properly, and this may be why the protein does not operate. Another point is what William Martin just mentioned. I think that we can learn a lot from the comparison between plastids, mitochondria and hydrogenosomes, and the gene rearrangement that we see in the eukaryotic cell. Now it is clear that if we translocate genes there is no great problem when we transfer genes for simple structures like single enzymes, but translocation of genes for complex structures can only be gradual, and it is unidirectional. Now this means that we have structures of dual genetic origin in plastids and we have in plastids probably more than half a dozen such structures, and in the most advanced mitochondria we only have two, the respiratory membrane and the ribosome, to make this respiratory membrane. In some hydrogenosomes, we do not need the respiratory membrane, so we do not need all of those ribosomes. This is probably one of the selective reasons why we do not have DNA in most hydrogenosomes. I do not think that it has anything to do with redox control, but this is really a very interesting question.

J. F. Allen (*Plant Biochemistry, Lund University, Lund, Sweden*). *Toxoplasma*, *Plasmodium* and *Epifagus* contain organelles that clearly were once chloroplasts and are now no longer, yet they retain genomes. Now do we know that these organelles do not have vectorial membrane electron and proton transfer? Can we be clear about that?

J. A. Raven. No, we do not. I think I have read something recently that suggests that there could be such a process going on in the apicoplast in relation to polyisoprene synthesis and modification.

J. F. Allen. This would be the prediction of the theory that the forces are still operating.

J. A. Raven. The force is still with us, as it were.

GLOSSARY

A: adenine
C: cytosine
G: guanine
ORF: open reading frame
RUBISCO: ribulose-1,5-biphosphate carboxylase–oxygenase
T: thymine
U: uracil